16. (Twice Amended) A retroviral vector according to claim 15 wherein the immunological molecule is an immunoglobulin.

17. (Twice Amended) A retroviral vector according to claim 16 wherein the second NS is a nucleotide sequence coding for an immunoglobulin heavy chain variable region.

18. (Thrice Amended) A retroviral vector according to claim 1 wherein the vector additionally comprises a functional intron.

19. (Twice Amended) A retroviral vector according to claim 18 wherein the functional intron is positioned so that it restricts expression of at least one of the NOIs in a desired target site.

21. (Thrice Amended) A retroviral vector according to claim 1 wherein the vector or pro-vector is a murine oncoretrovirus or a lentivirus retroviral vector or pro-vector.

22. (Twice Amended) A retroviral vector according to claim 21 wherein the vector is a MMLV, MSV, MMTV, HIV-1, or EIAV retroviral vector.

23. (Thrice Amended) A retroviral vector as defined in claim 1 wherein the retroviral vector is an integrated provirus.

24. (Thrice Amended) A retroviral particle obtained from a retroviral vector according to claim 1.

42. (Thrice Amended) A retroviral vector according to claim 1 wherein said retroviral vector differentially expresses NOIs in target cells.

REMARKS

I. Introduction

Applicants respectfully request reconsideration and withdrawal of the rejections set forth in the Final Office Action and sustained in the Advisory Action.

Claims 20, 25-26, 28-29, 31-41, and 43-45 have been canceled, without prejudice or disclaimer thereof. Applicants reserve the right to prosecute the subject matter of these claims in this or another application.

In addition, claims 1-19, 21-24, and 42 have been amended. Details of the amendments are provided in the following discussion. None of the amendments introduce new matter.

Upon entry of this Amendment, claims 1-19, 21-24, 30, and 42 will be pending in the application.

II. Summary of the Invention

For the Examiner's convenience, Applicants include the following brief summary of their claimed invention.

The claimed invention provides a retroviral vector-based gene delivery system that efficiently expresses one or more nucleotides of interest (NOIs) at one or more desired target sites. The invention also provides a system for preparing high titers of retroviral vectors. This system incorporates safety features for efficient *in vivo* expression of one or more NOIs at one or more target sites.

The safety features of the retroviral vector include splice donor/acceptor sites that only become active upon transduction, *i.e.* following reverse transcriptase activity. In the parent vector (the RNA stage), the splice donor/acceptor sites are rendered inactive by placing the splice donor site downstream of the splice acceptor site (in the 3' U3-R region). Translocation of the splice donor site to a location upstream of the splice acceptor site occurs via reverse transcription of the parent vector (the DNA stage). Transduction and insertion of the reverse transcribed DNA, which originates from the parent vector into the host cell genome, results in a pro-virus (DNA) that carries a functional intron containing a nucleic acid sequence of interest. However, because the gene of interest is bracketed by the intron, no transcription will take place from the gene due to splicing out of the sequence in mRNAs transcribed from the pro-virus. Moreover, in preferred embodiments, expression of a second gene of interest that is positioned downstream of the splice acceptor site is activated because of the functioning intron (see page 25, lines 4-13, of the application).

The claimed invention provides highly desired *in vivo* stable gene expression, rather than transient gene expression. Specifically, the invention's retroviral vector, which is generated from primary target cells, can transduce secondary target cells. Also, gene expression in the secondary target cells is stably maintained due to the integration of the retroviral genome into the host's DNA.

The secondary target cells do not express significant amounts of viral antigens and are therefore less immunogenic than cells transduced with adenoviral vector (see page 45, lines 5-12 of the application).

Moreover, use of the claimed invention's retroviral vector as a secondary vector enables targeting of specific cell types, such as rapidly dividing cells, as well as limited gene expression to a primary or secondary target site. This eliminates a NOI's possible toxicity or antigenicity (see page 45, lines 20-25, of the application).

III. Rejections under 35 U.S.C. § 112, 1st Paragraph

Claims 1-26, 28, 30, and 42 were rejected under 35 U.S.C. § 112, 1st paragraph, as allegedly not being enabled for any nucleotide of interest, as claimed. The Examiner did acknowledge, however, that the application enables the selective expression of the hygromycin-neomycin gene pair or the hygromycin-p450 gene pair. Additionally, the specification provides an enabling disclosure for other embodiments of the invention. For instance, Example 6 describes construction of a conditional expression vector for cytochrome P450 in which the coding sequences of the cytochrome P450 gene are arranged in two halves such that correct splicing to allow P450 expression occurs only upon transduction (see Figure 18).

A. Skilled Artisans Can Detect and Manage Cryptic Splice Donor/Acceptor Sites

As one basis for the enablement rejection, the Examiner asserted that the selective expression of foreign genes is unpredictable. In particular, the Examiner noted that cryptic splice donor/acceptor sites pose a potential challenge. Applicants respectfully traverse this ground for rejection.

Prior to the claimed invention, skilled artisans were aware of cryptic splice donor/acceptor sites, could readily detect them, and could readily eliminate them. Applicants enclose herewith copies of two articles evincing that cryptic splice/donor sites were known in the art: Sebillon *et al.*, *NAR*, 1995, 23:3419025 and Maruyama *et al.*, *Eur. J. Biochem.*, 1995, 232:700-05. Means for identifying such sites included performing sequence analysis, especially in those cases where transcripts of unexpected lengths were obtained. Prior to the claimed invention, those skilled in the art also knew that mutating cryptic splice sites leads to improved levels of gene expression. In 1996, Reichel *et al.* reported that removal of a cryptic splice site significantly improve GFP performance. Reichel *et al.*, *PNAS*, 1996, 93:5888-93. Similarly, Burn *et al.* reported in 1995

that the modification of a plasmid sequence to eliminate a cryptic splice event optimized the plasmid's performance. Burn *et al.*, *Gene*, 1995, 161:183-87.

In the Advisory Action, the Examiner dismissed these references as allegedly not correlating to the claims. While acknowledging that the references teach mutation of cryptic splice sites to improve expression, the Examiner asserted that they do not teach how to activate splice sites upon transduction in a retroviral vector or teach how to use the mutated sequences in combination with other NOI.

Applicants respectfully submit that the proffered references contain teachings sufficient to rebut the rejection. The rejection rests on the basis that cryptic splice sites might become active and prevent protein expression. In response, Applicants have established, through these several references, that prior to the application's filing date, detecting and mutating cryptic splice sites was routine, not unpredictable. Thus, Applicants properly excluded such matter from their specification because a patent preferably omits what is well known in the art. *In re Buchner*, 929 F.2d 660,661 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384 (Fed. Cir. 1986).

Now, the Examiner seeks further to require evidence of mutating cryptic splice sites in a retroviral vector, specifically. However, the enablement requirement of 35 U.S.C. § 112 does not require this specific teaching. "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without *undue experimentation.*" *United States v. Telectronics, Inc.*, 857 F.2d 778, 785 (Fed. Cir. 1988)(emphasis added). Applicants have established that mutating cryptic splice sites was routine at the filing date of their application. By virtue of being routine, mutating cryptic splice sites does not require undue experimentation. If the PTO now wishes to perpetuate the enablement rejection, it must present reasons for its continued uncertainty. *In re Bowen*, 492, F.2d 859, 862-63 (CCPA 1974); *In re Marzocchi*, 439 F.2d 220, 224 (CCPA 1971). This would include an explanation of why mutating cryptic splice sites in plasmid vectors (as used in the submitted references) does not relate to doing the same in retroviral vectors.

The Applicants respectfully submit that their argument and submission of state-of-the-art references overcomes the enablement rejection. Accordingly, they respectfully request its withdrawal.

B. Gene Therapy

As a second basis for the enablement rejection, the Examiner asserted that gene therapy is an unpredictable art. For the sole purpose of advancing the prosecution of this application, Applicants have canceled all claims directed to gene therapy. However, Applicants reserve the right to pursue the subject matter of these claims in this or another application. In addition to canceling the gene therapy claims, Applicants have amended claim 5 such that it no longer recites a "therapeutic agent." Therefore, the rejection is moot.

As amended, the claims are directed to a vector product, which has utility as a tool for delivering an NOI to a cell. The specification enables how to perform such delivery in more than one respect. For example, Example 2 describes a pIUXT vector containing both *Hygro* and *Neo* (see Figure 13), and a Hygro-p450 cMLV pICUT construct (see Figure 14). In this example, producer cells express only *Hygro*, while transduced cells express the pro-drug p450 2B6 isoform (see Figure 14). Furthermore, page 35, paragraphs 1-2 of the specification give guidance regarding a number of second NOIs useful in the invention. Many of these are routinely used in *in vitro* and *in vivo* therapeutic and diagnostic methods. They include sequences encoding cytokines, antibodies, fusion proteins, and enzymes. These and many other NOIs have the capacity to function in a therapeutic or diagnostic manner when carried by a vector. (See, *e.g.*, U.S. Patent Nos. 6,319,707; 5,672,344; 6,303,380; 6,207,648; 5,672,344; Kramm *et al.*, Hum. Gene Ther. 7: 291-300 (1996); Sewell *et al.*, Arch. Ololaryngol. Head Neck Surg. 123: 1298-1302 (1997); Cardoso *et al.*, Hum. Gene Ther. 4: 411-418 (1993); Podda *et al.*, PNAS 89: 9676-9680 (1992); Kenneth W. Culver, ed., Gene Therapy: A Handbook for Physicians, New York: Mary Liebert 1994, Chapter 6, pp. 63-66.

IV. Rejections under 35 U.S.C. § 112, 2nd Paragraph

Claims 1-26, 28, 30, and 42 were rejected under 35 U.S.C. § 112, 2nd paragraph, as allegedly being indefinite. The Examiner asserted that in claim 1, it is unclear how the "retroviral vector" correlates to the "retroviral pro-vector." The Examiner also asserted that nucleic acid sequences do not "encode" splice sites and that it is unclear how limitations describing the retroviral pro-virus limit the retroviral vector of claim 1.

Applicants have amended claim 1 to clarify the relationship between the retroviral vector and the retroviral pro-viral vector: the retroviral vector is formed as a result of reverse

transcription of a retroviral pro-vector. In the pro-vector, the 3' U3-R region contains a nucleotide sequence containing a splice donor site, such that a copy of the splice donor site is translocated to the 5' LTR of the transcribed retroviral vector. Given this relationship, it is apparent that limitations of the retroviral pro-vector will affect the retroviral vector. Applicants have also amended the claims to recite that the nucleic acid sequences contain, rather than encode, splice sites.

Because the amended claims are definite, Applicants respectfully request that the Examiner withdraw the rejection.

V. Rejections under 35 U.S.C. § 102

Claims 1-6, 9-25, and 43-44 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by three references: (1) Morgenstern *et al.*, Nucleic Acids Research, Vol. 18, No. 12, 3587 (1990); (2) Takeda *et al.*, Nature, Vol. 314, April 4, 1985, pp. 452-54; and (3) Kriegler *et al.*, Cell, Vol. 38, September 1984, pp. 483-491. Applicants respectfully traverse the rejections.

A. Morgenstern *et al.* Do Not Describe the Claimed Invention

Claims 1-6, 9, 10, 12-15, 18-25, and 43-44 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Morgenstern *et al.* According to the Examiner, Morgenstern *et al.* describe a retroviral vector comprising a functional splice donor site and a functional splice acceptor site, including the limitations of each rejected claim.

Contrary to the Examiner's assertion, Morgenstern *et al.* do not describe the claimed invention. Although Morgenstern *et al.* provide some vectors with functional splice donor sites (*e.g.*, pRLV S+), the splice donor sites are not positioned according to the recitations of claim 1. The specific positioning in the claimed retrovirus vectors represents an important safety feature that Morgenstern *et al.* lacks: it ensures that the retroviral vector's splice donor/acceptor sites only become active upon transduction (*see, e.g.*, specification, pg. 25, ln. 9-13). This directly contrasts with the vectors of Morgenstern *et al.*, which are direct orientation (DO) retroviral vectors.

Specifically, the retroviral vector of the claimed invention derives from a retroviral pro-vector that comprises a first nucleotide sequence (NS) capable of yielding a functional splice donor site and a second NS capable of yielding the functional splice acceptor site, wherein the first NS is positioned downstream of the second NS such that the retroviral vector is formed *only* as a result of

reverse transcription of the retroviral pro-vector. Reverse transcription of the pro-vector reshuffles the first and second NSs and arranges them in the correct, functional order. This rearrangement is a necessary prerequisite for retroviral vector formation before integration. Thus, there are both structural and functional relationships between the pro-vector and the integrated form of the retroviral vector of the claimed invention that Morgenstern *et al.* lacks.

As described, claim 1 requires that the retroviral vector, in which the functional splice donor site is oriented upstream of the splice acceptor site, be formed as a result of reverse transcription of the retroviral pro-vector, in which the splice donor site is positioned downstream of the splice acceptor site. To enable the reverse transcription of the pro-vector to reshuffle the first and second NSs and arrange them in the correct, functional order as described in the claims, it is implicit that the splice donor site in the pro-vector is positioned in the 3' U3-R region and that the splice donor site in the retroviral vector is present in the 5' LTR. None of the cited prior art documents describe vectors having these features. Nevertheless, to further clarify the claims, claim 1 has been amended to explicitly state that the splice donor site of the retroviral vector is present in the 5' LTR and that the retroviral vector is formed by reverse transcription of the retroviral pro-vector such that a copy of the first NS containing the splice donor site at the 3' U3-R region of the retroviral pro-vector is translocated to the 5' LTR of the retroviral vector.

The relative positioning set forth in claim 1 enables the vectors of the claimed invention to be used for splicing when the vector is transduced into a host cell genome (which can be advantageous, *e.g.*, in allowing splicing out of a packaging signal, allowing increased expression, *etc.*), but avoids undesired splicing when the vector is in virion RNA form (thereby avoiding the problem of low titer). The feature also significantly improves the *in vivo* utility of the retroviral vector.

This is in complete contrast to the general teachings of Morgenstern *et al.*, which relates to an efficient "splice deficient" vector (pg. 3588, col. 2, para. 1), which lacks splice donor sites in the positions recited by the present claims and does not impart the advantages associated therewith. Neither type of vector generated by Morgenstern *et al.* (those containing "splice deficient" nor those containing "splice functional" sites), requires reverse transcriptase activity to functionally align the splice donor and splice acceptor sites for achieving effective splicing. Furthermore, none of the vectors taught by Morgenstern *et al.* show a splice donor site in the 5' LTR region of the vector. For example, the retroviral vector prZNSV(X), to which the Examiner referred, has a

splice donor site ("S_D") between the U5 region of the 5' LTR and the *Hygro* gene (see figure 2). Similarly, in each of the other vectors having an S_D site, the S_D site is positioned between the 5' LTR and the 3' LTR regions (see figures 1, 3, 5, 6 and 7). Given this positioning, they cannot be derived from reverse transcription of a pro-vector in which the corresponding splice donor sequence was present at the 3'U3-R region. Therefore, Morgenstern et al. do not teach vectors having the physical features of claim 1.

Because Morgenstern *et al.* do not describe the claimed invention, Applicants respectfully request that the Examiner withdraw the rejection.

B. Takeda *et al.* Do Not Describe the Claimed Invention

Claims 1 and 15-17 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Takeda *et al.* According to the Examiner, Takeda *et al.* teach a retroviral vector that comprises a nucleic acid sequence encoding immunoglobulin genes and several splice donor/acceptor pairs.

In contrast to the Examiner's assertions, Takeda *et al.* do not teach the claimed invention. Specifically, Takeda *et al.* do not provide any regions encoding splice donor sites within a long terminal repeat (LTR) of the retrovirus. This is clear from Figure 1 of the reference, which shows that the insertion of a heterologous sequence did not disrupt the LTRs. Instead, the heterologous sequence was inserted between LTRs. Thus, the vectors taught by Takeda *et al.* do not have the features of the vectors of the claimed invention and do not provide the previously discussed advantages and safety features of the claimed invention.

Because Takeda *et al.* do not describe the claimed invention, Applicants respectfully request that the Examiner withdraw the rejection.

C. Kriegler *et al.* Do Not Describe the Claimed Invention

Claims 1 and 9-11 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Kriegler *et al.* According to the Examiner, Kriegler *et al.* teach retroviral vectors containing several splice donors/acceptors and the early genes of SV40, which includes the small t antigen.

In contrast to the Examiner's assertions, Kriegler *et al.* do not teach the claimed invention. As with Takeda *et al.*, Kriegler *et al.* do not provide any regions encoding splice donor sites within a long terminal repeat (LTR) of the retrovirus. This is clear from Figure 1 of the reference, in which the indicated splice donor sites are each present between the LTRs. More specifically,

Figure 1(d) of the reference shows that insertion of a heterologous sequence did not disrupt the LTRs. Instead, the heterologous sequence was inserted between LTRs. Thus, the vectors taught by Kriegler *et al.* do not have the features or advantages associated with the vectors of the claimed invention. Moreover, the approach taken by Kriegler *et al.* does not provide the previously discussed advantages and safety features of the claimed invention.

Because Kriegler *et al.* do not teach the claimed invention, Applicants respectfully request that the Examiner withdraw the rejection.

VI. Conclusion

The present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

If there are any fees due in connection with the filing of this Amendment, please charge the fees to our Deposit Account No. 19-0741. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Date: April 3, 2002

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Version with Markings to Show Changes Made

1. (Twice Amended) A retroviral vector [delivery system] comprising:

(a) a first nucleotide sequence ("NS") containing [encoding] a functional splice donor site; and

(b) a second NS containing [encoding] a functional splice acceptor site; wherein:

(i) the functional splice donor site and the functional splice acceptor site flank a first nucleotide sequence of interest ("NOI");

(ii) the functional splice donor site is within the 5' long terminal repeat ("LTR") of the retroviral vector and is upstream of the functional splice acceptor site; and

(iii) the retroviral vector is formed as a result of reverse transcription of a retroviral pro-vector, wherein the retroviral pro-vector comprises:

(a) a first nucleotide sequence ("NS") containing [encoding the] a splice donor site; and

(b) a second NS containing [encoding the] a splice acceptor site; wherein the first NS is at the 3' U3-R region of the retroviral pro-vector and is downstream of the second NS; such that the retroviral vector comprising [a] the first NS containing [encoding a] the functional splice donor site and [a] the second NS containing [encoding a] the functional splice acceptor site is formed as a result of reverse transcription of the retroviral pro-vector such that a copy of the first NS containing the splice donor site at the 3' U3-R region of the retroviral pro-vector is translocated to the 5' LTR of the retroviral vector.

2. (Twice Amended) A retroviral vector [delivery system] according to claim 1 wherein the retroviral pro-vector comprises a third NS that is upstream of the second NS; wherein the third NS contains [encodes] a non-functional splice donor site in the retroviral vector.

3. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the retroviral vector further comprises a second NOI; wherein the second NOI is downstream of the functional splice acceptor site.

4. (Thrice Amended) A retroviral vector [delivery system] according to claim 3 wherein the retroviral pro-vector comprises the second NOI; wherein the second NOI is upstream of the second NS.

5. (Thrice Amended) A retroviral vector [delivery system] according to claim 3 wherein the second NOI, or the expression product thereof, is [or comprises] capable of providing a therapeutic [agent] or a diagnostic [agent] effect.

6. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the first NOI, or the expression product thereof, is or comprises any one or more of an agent conferring selectability, a viral essential element, or a part thereof, or combinations thereof.

7. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the first NS is at or near to the 3' end of a retroviral pro-vector.

8. (Thrice Amended) A retroviral vector [delivery system] according to claim 7 wherein the first NS of the retroviral pro-vector comprises a third NOI; wherein the third NOI is any one or more of a transcriptional control element, a coding sequence, or a part thereof.

9. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the first NS is a viral NS.

10. (Twice Amended) A retroviral vector [delivery system] according to claim 9 wherein the first NS is an intron or a part thereof.

11. (Twice Amended) A retroviral vector [delivery system] according to claim 10 wherein the intron is the small t-intron of SV40 virus.

12. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the retroviral pro-vector comprises a retroviral packaging signal; and wherein the second NS is located downstream of the retroviral packaging signal such that splicing is prevented at a primary target site.

13. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the second NS is placed downstream of the first NOI such that the first NOI is expressed at a primary target site.

14. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the second NS is placed upstream of a multiple cloning site such that one or more additional NOIs may be inserted.

15. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the second NS is a nucleotide sequence coding for an immunological molecule or a part thereof.

16. (Twice Amended) A retroviral vector [delivery system] according to claim 15 wherein the immunological molecule is an immunoglobulin.

17. (Twice Amended) A retroviral vector [delivery system] according to claim 16 wherein the second NS is a nucleotide sequence coding for an immunoglobulin heavy chain variable region.

18. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the vector additionally comprises a functional intron.

19. (Twice Amended) A retroviral vector [delivery system] according to claim 18 wherein the functional intron is positioned so that it restricts expression of at least one of the NOIs in a desired target site.

21. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the vector or pro-vector is a murine oncoretrovirus or a lentivirus retroviral vector or pro-vector.

22. (Twice Amended) A retroviral vector [delivery system] according to claim 21 wherein the vector is a MMLV, MSV, MMTV, HIV-l, or EIAV retroviral vector.

23. (Thrice Amended) A retroviral vector [delivery system] as defined in claim 1 wherein the retroviral vector is an integrated provirus.

24. (Thrice Amended) A retroviral particle obtained from a retroviral vector [delivery system] according to claim 1.

42. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein said retroviral vector [delivery system] differentially expresses NOIs in target cells.